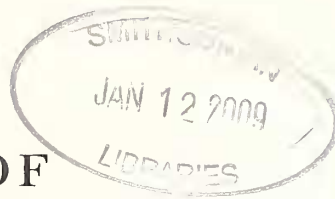


JOURNAL OF THE LEPIDOPTERISTS' SOCIETY



Volume 62

2008

Number 4

Journal of the Lepidopterists' Society
62(4), 2008, 177–188

MONARCH BUTTERFLY CLUSTERS PROVIDE MICROCLIMATIC ADVANTAGES DURING THE OVERWINTERING SEASON IN MEXICO

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ABSTRACT. Monarch butterflies form dense clusters in their overwintering colonies in the mountains of central Mexico, where forest cover provides protection from environmental extremes. We tested the hypothesis that the clustering behavior of the butterflies further moderates the microclimate they experience. We inserted hygrochrons (miniaturized digital hygrothermographs) into clusters for two-day periods during the 2006–07 and 2007–08 winters and compared temperature and relative humidity inside and outside the clusters. The inside of the clusters remained significantly warmer at night and significantly cooler during the day, with higher relative humidity during both day and night. Consequently, the butterflies inside the clusters may have gained some protection from freezing, reduced their rate of lipid burning, and lowered their rate of desiccation. The differences were small, but these studies were conducted during calm, moderate conditions, and the effects are likely to be more pronounced under more severe weather, including mid-winter storms and late season aridity. The microclimatic advantages of the monarchs' clustering behavior on fir boughs add to the known repertoire of the butterflies' overwintering adaptations to the high altitude environment that they occupy each year from November through March.

Additional key words: aggregation, insulation, clustering behavior, temperature, humidity.

Aggregation behavior is widespread in the animal kingdom and confers two major adaptive advantages to individuals: protection from predators and favorable modification of microclimate. Forming tight groups in many species of vertebrates and invertebrates reduces the probability, through the selfish herd effect, that any one individual will be killed (Hamilton 1971; Gamberale & Tullberg 1998). This advantage is enhanced when the

individuals are chemically defended (Brower 1984; Pough 1988; Sillen-Tullberg & Leimer 1988). The monarch butterfly (*Danaus plexippus* L., Lepidoptera, Danainae) is a classical example. The extreme densities of overwintering butterflies reduce the likelihood of any individual being attacked, as does their ability to store cardiac glycosides that are emetic to vertebrate predators (Brower *et al.* 1967; Brower 1984; Seiber *et*

al. 1986). These poisons reduce avian predation by reinforcing learned visual aversion of the butterflies' orange, black, and white warning coloration. Though substantial predation in the overwintering colonies in Mexico occurs by orioles and grosbeaks (Calvert *et al.* 1979; Fink & Brower 1981; Brower & Calvert 1985) and by certain species of mice (Brower *et al.* 1985), the majority of birds (Fink *et al.* 1983; Brower & Fink 1985) and mice (Glendinning & Brower 1990) are substantially deterred.

Microclimatic effects also influence aggregations, with animals often choosing sites where conditions are moderated. Numerous insects are known to respond to small differences in temperature and humidity (Cloudsley-Thompson 1962; Waldbauer 2000); for example, ladybird beetles and weevils aggregate where humidity is higher (Simpson & Welborn 1975), and cutworm moths aggregate in alpine talus (White *et al.* 1998), where temperatures are less extreme. Also, animals may create moderated conditions within their aggregations. For example, cockroaches and crickets generate higher humidity within their clusters (Dembach & Goehlen 1999; Yoder *et al.* 2002). Our study explored possible microclimatic advantages that

monarch butterflies derive from their clustering behavior.

One of the great biological spectacles on earth is the aggregation behavior of monarch butterflies at their overwintering sites in the Transverse Neovolcanic Range in central Mexico (Brower 1995). Arriving on at least twelve separate mountain massifs (Slayback *et al.* 2007; Slayback & Brower 2007) in early November, the butterflies form extremely dense clusters on the boughs and trunks of coniferous trees in colonies that, by mid-December, range in area from 0.01 to 6.14 hectares (Fig. 1). The largest combined area of monarch clusters occurred during the 1996–1997 overwintering season (Missrie 2004; Slayback *et al.* 2007), with an estimated combined total of 18 hectares of forest festooned with butterflies. Recent estimates indicate that there are at least 50 million butterflies per hectare (Brower *et al.* 2004), so that the 1996–1997 aggregations contained about 900 million monarchs.

Even though the overwintering area of monarch butterflies is south of the Tropic of Cancer, the 3000 m plus elevation of the mountains on which they form their colonies subjects them to freezing temperatures. Their greatest natural mortality occurs by freezing to



FIG. 1. Aerial photograph of the Piedra Herrada overwintering colony in an oyamel fir forest in the state of Mexico. In mid to late Dec 2006, this small colony occupied 0.27 ha (Rendon-Salinas *et al.* 2007). The butterflies likely avoid clustering in the tree tops in order to avoid freezing from exposure to the cold night sky. 13 Feb 2007.



FIG. 2. The density of clustering monarchs varies according to the foliage architecture of the tree species on which they settle. Note the exceedingly dense clusters on the oyamel fir (left foreground) and the much smaller ball-like clusters on the pine (right background). Photo taken in the Ojo de Agua ravine on Cerro Pelon in the state of Mexico, 13 Feb 2004.

death after northern rain and snowstorm incursions wet them, followed by plunging temperatures as the weather clears. One such storm in January 1981 was estimated to have killed more than 2.5 million monarchs in a Sierra Chincua colony (Calvert *et al.* 1983), and in January 2002 a major winter storm killed nearly half a billion monarchs across the overwintering region (Brower *et al.* 2004).

By forming their colonies in dense coniferous forests and by avoiding the tree tops, monarchs derive microclimatic protection from the forest canopy that acts as a blanket and reduces the rate of radiant heat loss to the sky (Calvert & Brower 1981). This blanket effect is dramatically demonstrated by large differences in both maximum and minimum daily temperatures inside the forest compared to nearby open areas (Brower & Calvert 1985). A second microclimatic advantage of the forest canopy is that it acts as a partial umbrella and helps to prevent the butterflies from getting wet during winter rain and snowstorms (Anderson & Brower 1996). These authors also discovered that overwintering monarchs can withstand freezing at body temperatures down to about $-8\text{ }^{\circ}\text{C}$, but their natural cryoprotection is substantially lost if their bodies become wet. When the forest is thinned, holes are punched in the blanket and umbrella, and both the thermal and sheltering advantages are diminished (Calvert *et al.* 1983).

Based on observations of the sites dating back to 1977, the three most utilized tree species are, in order of importance: the oyamel fir, *Abies religiosa* H. B. K. (Pinaceae), the smooth bark Mexican pine, *Pinus pseudostrobus* Lindl. (Pinaceae), and the Mexican cedar, *Cupressus lusitanica* Miller (Cupressaceae) (synonym of *C. lindleyi*; GRIN, 2007). The architectures of individual clusters are determined by the growth form of the boughs and needles of the tree species on which the butterflies settle (Figs. 2, 3A–C). Anderson & Brower (1996) found that butterflies inside fir clusters gain an important microclimatic advantage: they did not get as wet as those on the outside (Fig 4). The authors deduced that individuals within the clusters would more likely survive subfreezing temperatures.

This paper presents the results of field experiments begun in 2007 and repeated in 2008 designed to test the hypothesis that butterflies inside the clusters are insulated by those on the outside, with three possible microclimatic advantages. First, during lethal temperature drops, the butterflies inside may remain warmer. Second, during the day when temperatures climb, the inner butterflies may stay cooler, thereby preserving their lipid reserves. Lipids are critical both for winter survival (Masters *et al.* 1988) and for the surviving monarchs' spring remigration back to the Gulf

Coast (Malcolm *et al.* 1993). Third, the butterflies on the inside of a cluster may enjoy higher humidity, thus reducing evaporation and desiccation, which intensify as the dry season advances and millions of monarchs engage in long to-and-fro flights to drink water.

MATERIALS AND METHODS

Location of the study sites. The colonies studied in both years were located in the Sierra Chincua massif in Michoacan, Mexico. Their coordinates were determined using a Garmin-CS GPS unit and the Anganguero topographic map (INEGI 1999). On 8 January 2007, the position of the colony near its upper boundary was $100^{\circ} 17' 58''\text{W}$, $19^{\circ} 40' 31''\text{N}$, at an elevation of 3256 m. This is at the head of the western-most tributary leading down into the Arroyo Hondo. On 5 February 2008, the colony was located 1.1 km to the east of the 2007 site, slightly east of the eastern-most tributary of Arroyo Hondo, at approximately $100^{\circ} 17' 19''\text{W}$, $19^{\circ} 40' 06''\text{N}$, at an elevation of 3317 m. Both of these areas have hosted overwintering colonies in almost exactly the same positions as reported nearly 30 years ago and in numerous overwintering seasons since then (Calvert & Brower 1986; Missrie 2004).

Hourly temperature and humidity data on the same dates were recorded by an electronic weather station (WeatherHawk, Model 232, Logan, UT) located on the Monarch Butterfly Biosphere Reserve (MBBR) Field Station on El Llano las Papas ($100^{\circ} 16' 6.2''\text{W}$, $19^{\circ} 39' 41.9''\text{N}$, elevation 3160 m). The field station is on the eastern edge of the Sierra Chincua in an open llano (field) adjacent to an oyamel fir forest. It is approximately 3.6 km ESE of our 2007 experimental site, and approximately 2.5 km ESE of our 2008 site. The WeatherHawk recorded temperature each hour averaged over the previous hour. All data were downloaded into spreadsheets for analyses. A hygchron attached to the underside of the weather station provided a direct comparison to the measurements of the other hygchrons used in the experiment.

Temperature and humidity measurements of the clusters. For successive nights in both 2007 and 2008, we measured temperature and relative humidity inside and immediately outside monarch clusters that had assembled on the boughs of oyamel fir trees within the Chincua colony. The recording devices (Fig. 5) were iButton Hygchrons (model DS1923, Dallas Semiconductor Corporation), which are small electronic disks (1.59 cm by 0.64 cm). The hygchrons were set to record an instantaneous reading once every twenty minutes.

For 2008, the hygchrons were evaluated by



FIG. 3. (a) An early winter cluster of monarchs on an oyamel fir, likely the optimal tree species substrate for the butterflies to hold onto and form extremely dense, bag-like clusters having a large volume to surface area ratio. Photo taken in the Sierra Chincua colony in the state of Michoacan, 9 Dec 2006.

comparing their readings under identical conditions. All hygrometers were placed in the same plastic bag to record temperature and humidity every 20 min during 13 hours of warm, room-temperature conditions (40 records) and 10 hours of cold, refrigerated conditions (30 records). We compared the average of the test readings for every pair of hygrometers used in an inside-outside comparison across a cluster. When the average reading of one hygrometer under test conditions was less than the other, that difference was added to the measurements from the field of the first hygrometer. We applied adjustments to the field data separately for day measurements (adjustments from the warm readings) and night measurements (adjustments from



FIG. 3. (b) An early winter cluster on a cedar tree, which has flatter needles and is likely a less optimal substrate for dense clusters than the fir. Photo taken on the Llano de los Tres Gobernadores colony, on Cerro Pelon in the state of Mexico, 11 Dec 2006.

the refrigerated readings). These sensors are advertised as having an accuracy of ± 0.5 °C and a resolution of 0.6% RH. We did not compare their accuracy against known standards, but by our measurements, the hygrometers gave such little variation in their readings that, in comparing them, we found the S.D. of the differences in temperature of each pair to range from only 0.03 to 0.07 °C. That meant that each sensor gave highly consistent readings and that, with precision, paired hygrometers could measure differences of less than 0.1 °C. Relative humidity readings were more variable, with S.D. of all pairwise differences ranging from 0.58 to 0.97%.

Inserting the hygrometers into the clusters.

Four (2007) or three (2008) hygrometers were attached with ©Velcro to 89 cm long by 0.95 cm diameter wooden dowels at approximately 20 cm intervals. The end of a #18 twisted nylon twine leading off a spool was then attached with duct tape to the top of the hygrometer dowel. To lift the string that was attached to the hygrometer dowel, we used a 3 m extensible pole to which a second dowel with a bent hook nailed into its end was taped. We raised the pole so that the hygrometer dowel attached to the string hung directly over the cluster center. By gently playing out the string through the hook to avoid disturbing the cluster, the dowel was lowered into the cluster center. Once vertically positioned, we carefully twisted the pole to release the string from the hook and then secured the string to hold the dowel in place with at least one hygrometer inside and one outside the cluster (Fig. 5).

Experiments. The goal was to compare the temperature and relative humidity inside and immediately outside the monarch clusters. In 2007,



FIG. 3. (c) A late winter cluster on a pine tree, the least favorable of the three major coniferous substrates for dense clusters. The ball-like pine clusters are smaller than those that form on the firs and cedars, thus providing less microclimatic protection. Photo taken in the Ojo de Agua ravine, 9 Mar 2006.



FIG. 4. Monarchs clustering on oyamel fir branches wetted by an early December storm. The small silverish spots are water drops. Note that the fir boughs provide an umbrella effect and that there are few raindrops on the butterflies. This microclimatic effect is greater in larger clusters where the butterflies inside the cluster have less or no water on them. Photo taken in the Sierra Chincua Arroyo Hondo colony in the state of Michoacan, 9 Dec 2006.

preliminary studies were run on two clusters (A1, A2), while also positioning a single outside hygrochron on a dead oyamel tree branch less than 10 m away (A3). The dowels were in place from 1540 on 8 Jan 2007 to 0940 on 10 Jan 2007. We used binoculars to confirm that the dowels maintained their positions inside the clusters throughout the experiment.

We repeated the experiment in February 2008, placing dowels with sensors into six clusters (B1–B6). To obtain repeated ambient measures inside the colony, three control hygrochrons were attached to another dowel (B7) that we hung from an oyamel tree branch on the western edge of the colony at about the same height as the study clusters. Three dowels (B1–B3) were in place from 1700 on 5 Feb to 1530 on 7 Feb, and an additional three dowels (B4–B6) were in place from 1200 on 6 Feb to 1500 on 7 Feb. The data from one cluster (B2) were later deleted from the analysis because butterflies subsequently surrounded all the hygrochrons, so there was no inside-outside comparison. The five other dowels yielded readings for two days (10 day-time comparisons), while two dowels



FIG. 5. The experimental dowel inserted into experimental cluster 2 on an oyamel fir bough on 9 Jan 2007. The bottom of the dowel with an exposed hygrochron is evident; the other three hygrochrons are inside the cluster. The inset is a closeup of a hygrochron attached to a dowel with Velcro.

recorded for two nights and the other three for a single night (7 night-time comparisons).

The hygrochrons recorded temperature and relative humidity every 20 min, but for analysis, we standardized the times for comparison as day, 1200–1700, and night, 0000–0800. These were the times recorded by the ambient hygrochrons as being the warmest and coolest periods of a 24 hour day and thus the times when insulating of the clusters would be the most important.

We also recorded wind speed in the colony during the 2008 experiment with a Wind Speed Smart Sensor attached to a HOBO Micro Station (Onset Computer Corp.). This instrument yielded the average and maximum wind speed during each five-min time block from 1800 on 5 Feb 2008 to 1430 on 7 Feb 2008.

Description of the clusters. Qualitative observations indicated that there were fewer large clusters during both overwintering seasons than has been the case in the past, and they were less dense than in most previous years. Daytime temperatures were high enough that care was necessary not to disturb the butterflies and cause them to "explode" out of the clusters. Over the course of the 2007 experiment, the sky was partly cloudy, and the sun shone occasionally on the clusters. One 2007 cluster (A2) diminished somewhat through

time because the colony was gradually moving down the arroyo, which is typical with the advance of winter (Calvert & Brower 1986). In 2008 the weather was clear throughout the experiment, and the clusters did not change in size during the course of the experiment.

Analyses. We performed statistical analyses with SPSS 14.0 (SPSS Inc.) separately for each year. Comparisons of the measurements inside and outside each cluster were made by one-tailed paired t-tests, with arcsin transformation of relative humidity data, and the results were evaluated with a modified Bonferroni correction for multiple tests (Walsh 2004). Error bars used in the figures are 95% C.I. about the means (± 1.96 S.E.). Data from both years were analyzed identically except that calibration of the hygrochrons for the 2008 measurements ensured that pairwise comparisons of their readings were more accurate.

RESULTS

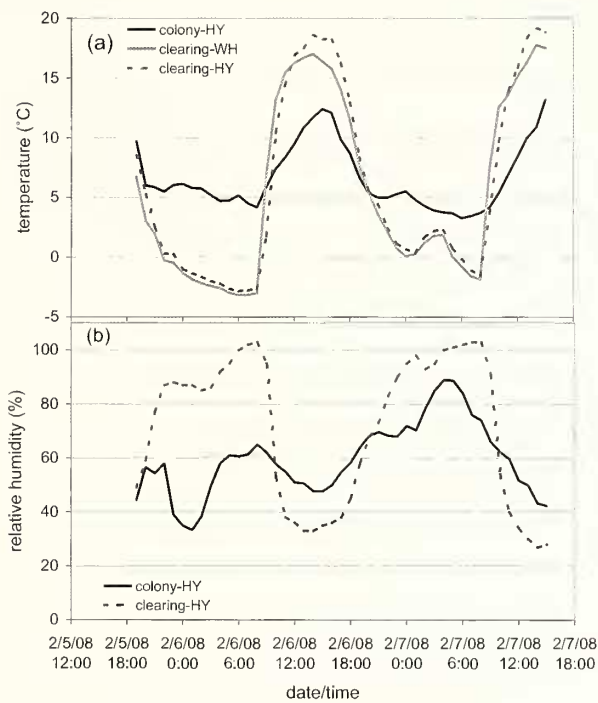


FIG. 6. Records of (a) temperature and (b) relative humidity from within the colony (average of three ambient hygrochrons) and from a clearing at the MBBR Field Station on the Llano de las Papas, Sierra Chineua, Michoacan, Mexico. The records from the clearing are given as recorded by both the Weather-Hawk weather station (WH; temperature only) and by a hygrochron attached to the weather station (HY). Data were recorded 5–7 Feb 2008 during three clear days. The variation in temperature and RH is much less within the colony than in the clearing, and the inverse relationship between temperature and RH is apparent. Humidity in the clearing ranges from 100% during the night to a drying 27% during the day.

Weather. During the 2008 experiment, records from the nearby Chincua weather station (Fig. 6) revealed a much greater range in temperature and RH (from -3.2° to 17.8°C and 27% to 100%) than was recorded in and around the monarch clusters ($+3.3^{\circ}$ to 13.2°C and 33% to 89%), which were in dense forest and thus less exposed. With the absence of precipitation during the very clear three days of recording, data measured at the weather station showed temperature and relative humidity to be inversely proportional (Fig. 6 a, b), as expected. Wind speed within the colony during our study gave five-minute averages up to 2.7 m/s, with gusts up to 3.8 m/s. Wind was highest during the afternoon, but even at night, wind was consistently more than 1.0 m/s.

2007 Experiment. Following the initial experiment in 2007, measurements of temperature and relative humidity were analyzed without calibration, and the results suggested microclimatic buffering within the clusters. The inside of cluster A1 remained significantly warmer at night ($t=6.491$, $df=49$, $p<0.001$), although this night-time difference did not hold for cluster A2. The differences in RH at night were mixed. Microclimatic buffering was conspicuously greater, however, in the daytime. Both clusters remained significantly cooler inside than outside by up to 0.3°C (Fig. 7; cluster A1: $t=7.682$, $df=22$, $p<0.001$; cluster A2: $t=3.879$, $df=20$, $p=0.001$). Coinciding with lower temperatures, both clusters also maintained significantly higher humidity inside (Fig. 8; cluster A1: $t=1.903$, $df=22$, $p=0.035$; cluster A2: $t=3.270$, $df=20$, $p=0.004$). The separate ambient sensor (A3) recorded up to 0.7°C colder temperatures at night and morning than did the

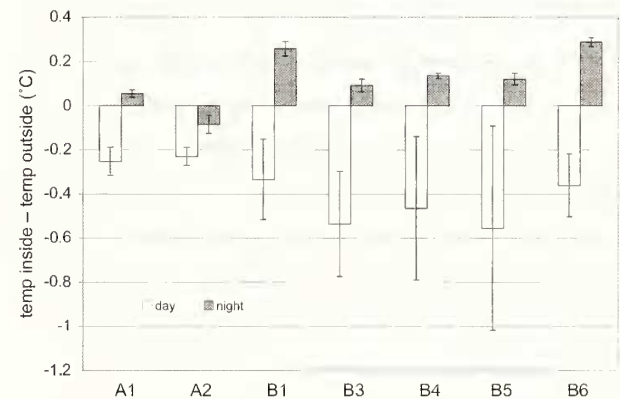


FIG. 7. Differences in temperature across the clusters. The inside minus the outside temperature is shown, averaged over all readings for each separate cluster, with error bars indicating the 95% CI for the means. The two 2007 clusters (initial experiment) are labeled A, and the five 2008 clusters are labeled B. In all cases, the inside of the clusters remained significantly cooler than the outside during the day (open bars), while 6 of the 7 clusters were significantly warmer at night (shaded bars).

sensors on the outside of the clusters, whereas it recorded up to 0.3°C warmer temperatures during the afternoon and evening. Even though thermal buffering was greater inside the clusters, the outside of the clusters experienced slightly more moderate conditions than ambient temperatures closer to the forest floor.

2008 Experiment. Before analyzing the 2008 data, we calibrated the hygrochrons separately for warm and cold temperatures, with readings of one hygrochron adjusted to match the measurements from the lab tests of the other hygrochron. The precision of the temperature readings was higher than that of the relative humidity readings. Temperature adjustments for the five hygrochron pairs ranged from 0.06° to 0.11°C for warm (day) data and from 0.11° to 0.16°C for cold (night) data. Adjustments for relative humidity ranged from 0.35% to 0.55% in warmth and from 0.93% to 1.07% in cold.

Using these calibrated measurements, the five clusters gave consistent results over the two days of

measurement (Figs. 7, 8). During the cold night hours, the inside of the clusters was significantly warmer than the outside for all five clusters (and six of the seven night measurements, with the seventh showing the same trend, Table 1). The difference between the inside and the outside declined during the long night hrs (Fig. 9). Three clusters (over four separate night comparisons; Table 1) had significantly higher RH inside despite the warmer temperatures, which would usually lead to lower RH. One cluster (B1) recorded lower RH, while there was no difference in another (B4).

As with the 2007 results, microclimatic effects were stronger during the warm afternoon hours. During daytime, the inside of the clusters remained significantly cooler than the outside for all five clusters (and eight of the ten separate comparisons, with the other two showing the same trend, Fig. 8, Table 1). Also, the inside of the clusters maintained significantly higher RH than the outside for all five clusters (and nine of the ten separate comparisons, a response reciprocal to that of

TABLE 1. Statistical results of all 2008 measurements, showing comparisons of the outside and inside readings of temperature and relative humidity from each monarch cluster. The comparisons for each of the five clusters (B1, B3, B4, B5, B6) have been separated for each day and each night in this table. Two days and two nights were analyzed for each cluster, except for clusters B4–B6, for which data were available for a single night. Analysis by paired t-tests was evaluated with modified Bonferroni correction for each set of comparisons.

| Comparison | | | Temperature | | | | Relative Humidity | | | | | |
|------------|--------|------------------------------|----------------|------|------|------|------------------------------|----------------|------|------|------|--|
| cluster | date | location of highest readings | t _x | d.f. | P | sig. | location of highest readings | t _x | d.f. | P | sig. | |
| day/warm | | | | | | | | | | | | |
| B1 | 6Feb08 | outside | 3.066 | 15 | .008 | ° | inside | 2.777 | 15 | .014 | ° | |
| B1 | 7Feb08 | outside | 3.054 | 8 | .016 | ° | inside | 4.588 | 8 | .002 | ° | |
| B3 | 6Feb08 | outside | 5.139 | 15 | .000 | ° | inside | 4.577 | 15 | .000 | ° | |
| B3 | 7Feb08 | outside | 9.702 | 8 | .000 | ° | inside | 4.135 | 8 | .003 | ° | |
| B4 | 6Feb08 | outside | 4.012 | 15 | .001 | ° | inside | 3.795 | 15 | .002 | ° | |
| B4 | 7Feb08 | outside | 2.075 | 8 | .072 | n.s. | inside | 3.243 | 8 | .012 | ° | |
| B5 | 6Feb08 | outside | 3.423 | 15 | .004 | ° | inside | 1.808 | 15 | .091 | n.s. | |
| B5 | 7Feb08 | outside | 2.135 | 8 | .065 | n.s. | inside | 3.128 | 8 | .014 | ° | |
| B6 | 6Feb08 | outside | 4.768 | 15 | .000 | ° | inside | 4.802 | 15 | .000 | ° | |
| B6 | 7Feb08 | outside | 6.097 | 8 | .000 | ° | inside | 4.302 | 8 | .003 | ° | |
| night/cold | | | | | | | | | | | | |
| B1 | 6Feb08 | inside | 16.749 | 27 | .000 | ° | outside | 5.547 | 27 | .000 | ° | |
| B1 | 7Feb08 | inside | 7.346 | 27 | .000 | ° | outside | 2.697 | 27 | .012 | ° | |
| B3 | 6Feb08 | inside | 2.511 | 27 | .018 | ° | inside | 3.888 | 27 | .001 | ° | |
| B3 | 7Feb08 | inside | 1.452 | 27 | .158 | n.s. | inside | 3.013 | 27 | .006 | ° | |
| B4 | 7Feb08 | inside | 23.991 | 27 | .000 | ° | same | 0.352 | 27 | .727 | n.s. | |
| B5 | 7Feb08 | inside | 7.544 | 27 | .000 | ° | inside | 5.651 | 27 | .000 | ° | |
| B6 | 7Feb08 | inside | 26.071 | 27 | .000 | ° | inside | 13.551 | 27 | .000 | ° | |

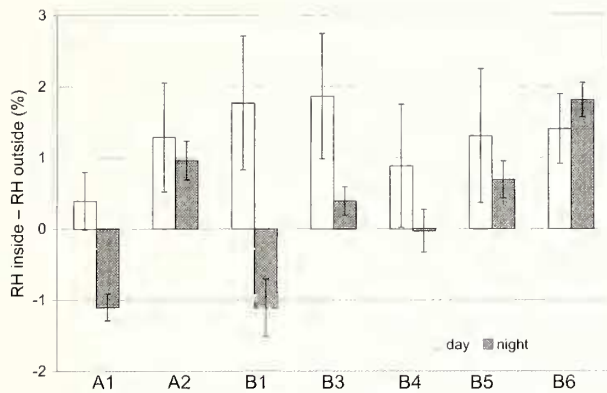


FIG. 8. Differences in relative humidity across the clusters. The inside minus the outside RH is shown, averaged over all readings for each separate cluster, with error bars indicating the 95% CI for the means. The two 2007 clusters (initial experiment) are labeled A, and the five 2008 clusters are labeled B. In all cases, the inside of the clusters remained more humid than the outside during the day (open bars), while 4 of the 7 clusters were significantly more humid at night (shaded bars).

temperature (Fig. 9, Table 1). The sensors in the control bough (B7) averaged 0.13°C warmer during the 12 hrs of day and 0.11°C warmer during the 12 hrs of night than the outside of the boughs with monarchs. These small differences suggest that the conditions immediately outside of the clusters were accurate representations of the ambient conditions at the same height within the forest.

DISCUSSION

The results found in 2008 support those suggested by the 2007 data: monarchs on the inside of clusters experienced warmer temperatures at night, cooler temperatures during the day, and elevated relative humidity throughout both day and night.

The coldest temperatures occur during night and early morning hours, so these are the times when microclimatic buffering against freezing comes into play. Significant buffering against cooler temperatures occurred throughout the 0000–0800 hr night period. Insulation against freezing would be most important for the butterflies in the clusters during the coldest moments, which occur when cloud cover opens up after winter storms and when cold air flows through the colony. While a difference of 0.1° to 0.2°C will not substantially affect the probability of monarchs freezing when they are dry, a combination of wetness and freezing temperatures during and immediately after winter storms strongly lowers their survivorship (Anderson & Brower 1996; Brower *et al.* 2004). Denser clusters, which frequently occur in years with larger

colonies, would likely increase the insulative effect.

Thermal buffering was stronger during daylight hours, with experimental clusters remaining cooler on the inside during peak warmth. Some variation exists among clusters because of different exposure to sunlight. The temperature differences are small, ranging up to 0.6°C; however, by lowering the warmest temperatures, these differentials may reduce metabolic rate by approximately 6.4% and the consequent consumption of critically limited lipid reserves (Masters *et al.* 1988). We estimated the lipid savings by assuming: (1) the empirical relationship between body temperature and metabolic rate as measured for adult California monarchs (Chaplin & Wells 1982); (2) an average weight for an overwintering butterfly of 530 mg (Calvert & Lawton 1993); (3) a temperature reduction of 3.6 degree-hrs per day (equivalent to 0.6°C for 6hr); (4) a 150 day overwintering period; and (5) the calm early February conditions under which this study was conducted. With these assumptions, the lipid savings for the overwintering season were small, ranging from 2 mg in a cold winter to 4 mg in a warm winter. These savings are in context of the average lipids in November being 129 mg per butterfly (unpubl. data). However, as ambient temperatures rise in late February and March, the thermal insulation of the inside of clusters may increase and thus produce greater lipid savings. Also, even small savings could affect those monarchs that arrive low in lipids by providing them with critical energy that they need to fly to water and to remigrate at the end of the overwintering season. A savings of a few mg of lipids could have a significant effect on survival.

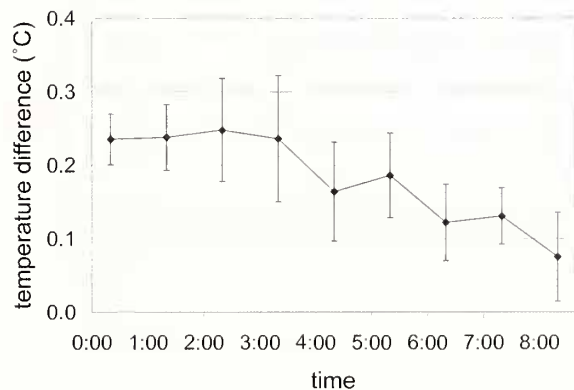


FIG. 9. Temperature difference between the inside and outside of the clusters through the night (0000–0840 hrs). The inside minus the outside temperature is shown, with error bars indicating the 95% CI for the mean each hour; the data show the average difference for three measurements each hour (e.g., 0000, 0020, and 0040 combined for 0020 hr) across all five 2008 clusters, with measurements recorded during 2 nights for clusters B1 and B3 and for 1 night for clusters B4–B6 (n=21 for each data point). The difference decreased by morning.

Relative humidity was higher by up to 3% inside all clusters during the day and higher at night in most, despite the temperature also being higher on the inside. Increased humidity reduces the threat of desiccation, an ever-present hazard when available moisture is limited, as is the case in the overwintering habitat as the dry season progresses. Part of the elevated humidity could have been due to evaporative transpiration from the fir needles within the butterfly clusters.

A greater range of temperature and relative humidity was found outside the clusters than inside. It is striking that structures as thin and seemingly delicate as butterfly wings provide insulation against environmental fluctuations, but when many wings are grouped together densely, as in the overwintering monarch colonies, the reason becomes clear. Still air is such a highly efficient thermal insulator that most heat exchange occurs through convective air movement, rather than through conduction. The microclimatic buffering in butterfly clusters derives from their wings trapping pockets of air that remain still, an effect that may have been supplemented by the fir bough needles. Single layers of butterflies serve as baffles that slow cross-wise air movement, while dense, multilayer clusters produce a quilt-like layer of insulation that blocks the convective exchange of heat between the outside and inside of a cluster. This effect would likely be even stronger during unstable weather when their wings also block winds.

Our results are based on comparisons of temperature and relative humidity inside and immediately outside the monarch clusters, and, as such, they do not distinguish potential microclimatic buffering provided by the fir needles from that created by the butterflies. It is likely, however, that the effect of the bough *per se* is less than the effect of the butterflies because most heat exchange is by convection, and air movement would be restricted more by a dense mass of butterfly wings than it would by an open bough of needles. The bulk of the microclimate differences inside and outside the clusters was likely from insulation produced by the densely packed butterflies, perhaps supplemented by buffering by the fir needles.

It is likely that the microclimatic advantages of clustering are diminished by even moderate forest thinning that results in colder nights (Calvert *et al.* 1984) and very likely warmer days. Unfortunately, illegal forest thinning, clear cutting, and burning of the clear cuts have become increasingly widespread in the Monarch Butterfly Biosphere Reserve (Brower *et al.* 2002; Ramirez *et al.* 2003, 2006; Honey-Roses & Galindo 2004; WWF-Mexico 2006; Brower *et al.* 2008).

It is also likely that larger clusters provide greater

microclimate protection of the butterflies than smaller ones. During the 1990's, one of us (LPB) witnessed enormously dense clusters in the Cerro Pelon colony, but has not seen such densities for several years. If the numbers of monarchs overwintering in Mexico continue to decrease, as is suggested by data from the last 15 yr (Rendon-Salinas *et al.* 2008), the average densities and cluster sizes may diminish along with a substantial measure of the microclimatic advantages of clustering that we have demonstrated.

CONCLUSIONS

Our results support the hypotheses that the clustering behavior of monarch butterflies on tree branches in their overwintering aggregations provides them with three microclimatic advantages, possibly enhanced by the fir boughs themselves: (1) buffering against lower temperatures during cold nights, thus lowering the probability of the butterflies inside the clusters freezing; (2) buffering against heating during warm days, thus reducing the rate at which the internal monarchs consume their lipid stores; and (3) maintaining higher humidity inside the clusters, thus lowering the rate of desiccation of the butterflies. While small, each of these factors contributes to a constellation of microclimatic advantages of clustering.

This study took place under moderate weather conditions. When clearing follows wet winter storms, however, the temperature inside the forest can plunge to as low as -5°C (Calvert *et al.* 1983), which leads to extensive mortality (Brower *et al.* 2004). Had this experiment been done under these conditions, it is likely that the magnitude of the temperature differences inside and outside the clusters would have been greater. Likewise, the advantage of clustering in maintaining higher humidity will most certainly be greater as the dry season advances and the weather becomes increasingly warm and dry.

The architecture of the short needled oyamel fir branches allows the butterflies to consolidate into larger and more dense bag-like clusters than possible on the flat needled cedars or the long needled pines (Figs. 2, 3). Because of the microclimatic advantages of clustering on boughs, there may be competition among individuals to position themselves toward the center of the clusters. More detailed studies of cluster architecture, butterfly clustering behavior, and possible microclimate advantages enhanced by the tree species upon which the butterflies form their clusters are needed.

ACKNOWLEDGEMENTS

We thank Stuart Weiss for suggesting the use of iButton

hydrochrons and Ivan Limon, Mia Magruder, and Carlos Carrillo Tellez for help in conducting the field experiments. We are grateful to Ing. Concepcion Miguel Martinez, Director of the Monarch Butterfly Biosphere Reserve, for facilitating our access to the colonies and to the Arizmendi family for providing accommodations in Angangueo. We are grateful to Myron Zalucki and two anonymous reviewers for critiques of the manuscript. We thank Jen Borton and Doug Weldon for discussion of the analyses and Tonya Van Hook for help with calibrating the hydrochrons. Diana Garland helped with editing the digital color images, taken by Lincoln Brower with a Canon D-20 camera. We also thank Lighthawk, Inc., and pilots Chuck Schroll and David Kunkel for providing aerial reconnaissance allowing the photography of the monarch colonies. Finally, we thank James Anderson for insights into the design of this experimental study. Support was provided by National Science Foundation grant DEB-0415340 to Sweet Briar College, with Lincoln Brower and Linda Fink as principal investigators, the October Hill Foundation, and the Monarch Butterfly Sanctuary Foundation. Ernest Williams was supported by the Leonard C. Ferguson Professorship Fund at Hamilton College. DGAPA-PA-PIIT (IN114707) provided financial support to M. Isabel Ramirez.

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Received for publication 6 Jun 2008; revised and accepted 12 Sep 2008